# Microgravity Crystallization of Protein Therapeutics Example: Interferon alfa-2b

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## **Outline**

- Background
  - Pharmaceutical Applications for Crystalline Proteins
  - Interferon alfa-2b (Biology and crystallization)
  - Microgravity Effects
  - SPRI –UAB/CBSE Connection
- Microgravity Studies: Results to date
  - Hardware development
  - Results from STS-51,70, 101 and 107
- Summary

## Pharmaceutical Applications for Crystalline Proteins

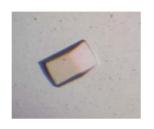
#### Area

Drug Discovery

#### Manufacturing

Product Development

#### Goal

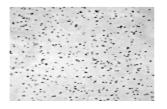


#### <u>Application</u>

- -Structure determination
- -Structure assisted drug design

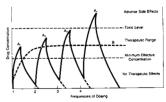






- -Purification (interferon)
- -Storage / stability

-Controlled Release (insulin)



-Pulmonary Delivery (interferon) (no injections)

# Interferon Alfa-2b (Background)

#### **Biology**

- Interferon alpha-2b: human protein possessing potent antiviral and immuno-modulatory activity
- An approved therapeutic for hepatitis B & C, melanoma, venereal warts, AIDS related Kaposis sarcoma and hairy cell leukemia
- Recombinant protein marketed as INTRON A and a pegylated version marketed as PEG-INTRON A

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Application Structure determination	Morphology cubic	Method zinc complex /temperature
Purification	needle	temperature
Controlled release	cubic	zinc complex /temperature
Pulmonary Delivery	spherulite	temperature

#### Microgravity Effects and Challenges

#### **Theoretical Advantages:**

- Minimize sedimentation effects
- Minimize density gradients
- Control convection currents

#### **Challenges:**

- Cost
- Accessibility (launch delays, scrubs, flight opportunities)
- Experimental access (limited monitoring)
- Recovery

Overall unique research tool!

## SPRI-UAB/CBSE Connection

Goal: produce microgravity derived crystalline IFN suspensions and compare to ground based experiments using biological and physical biochemical methods

University of Alabama at Birmingham Center for Biophysical Sciences and Engineering (UAB/CBSE):

Experience/ hardware/ processing/ integration/ analysis/ human resources

Schering Plough Research Institute(SPRI): Protein / experimental design/ analysis/ human resources

Both proprietary and non-proprietary experiments

### Protein Crystallization Facility (PCF) Hardware





Large scale- temperature induced batch crystallization

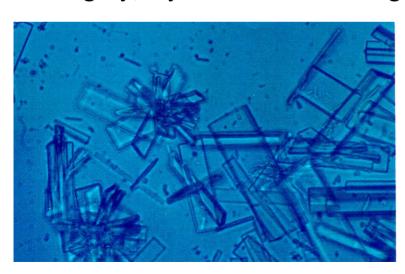
- Polysulfone bottles (1- 1000 ml capacity) with aluminum tops
- Thermal carrier: C-RIM (flight incubator)
- SPRI-UAB/CBSE hardware patent
- Crystallization studies alpha-2b interferon using (pI crystallization method; 4 to 22 ° C over 24 -48 hour gradient)
- Large scale Zinc- interferon complex crystallization method was developed
- Goal: crystals suitable for structure determination

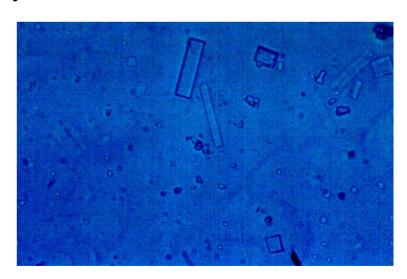
# Microgravity IFN Crystallization Studies: STS-51



Analysis of Zinc -interferon crystalline protein from flight experiment:

- Retained full biological activity
- 2.4-fold increase in crystal dimensions was found in space-grown crystals
- 95% IFN crystallized
- Space-grown crystals remain a free flowing suspension
- Sample processing and storage in polysulfone bottles did not affect protein integrity, crystallization or biological activity

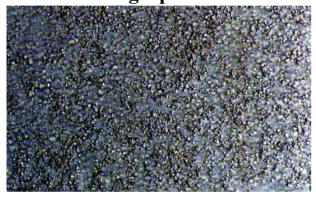




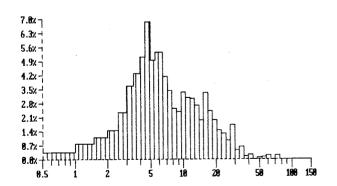
# Microgravity IFN Crystallization Studies: STS-70



Ground Control Experiment Photomicrograph 200x



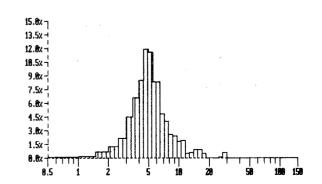
Particle size analysis:
Probability area density graph



Flight Experiment
Photomicrograph 200x

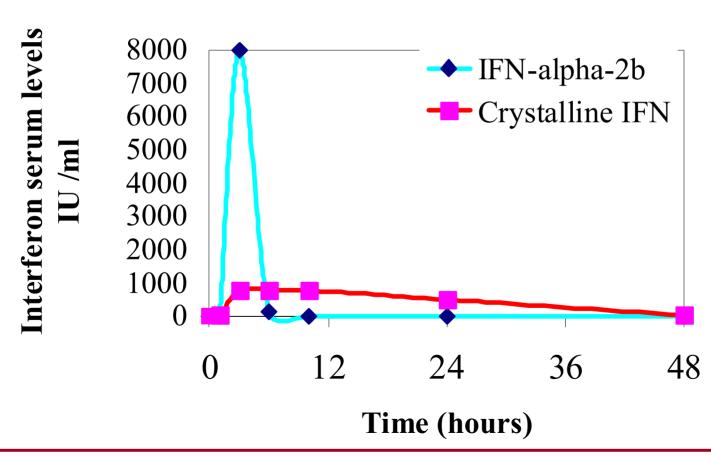


Particle size analysis:
Probability area density graph

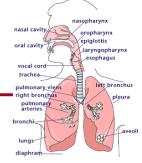


# Crystalline Interferon for Controlled Release STS-70 (Zinc IFN suspension)





# Crystalline Interferon Suspension for Pulmonary Delivery

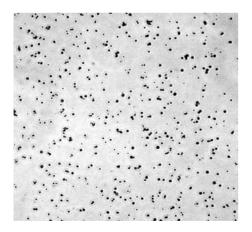


Rationale for pulmonary delivery (systemic delivery of interferon via pulmonary disposition)

Key for delivery: aerosol particle size (ca. 1 micron)

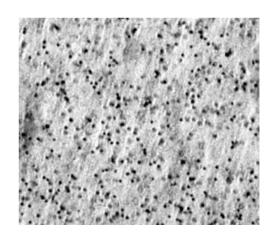
- Pulmonary delivery demonstrated for insulin and human growth hormone
- > Challenge to produce uniform crystalline interferon suspension suitable for pulmonary delivery in a pharmaceutically acceptable buffers via nebulizer

400X Photomicrograph / IFN suspension



mean size;  $0.78 \pm 0.2$  uM

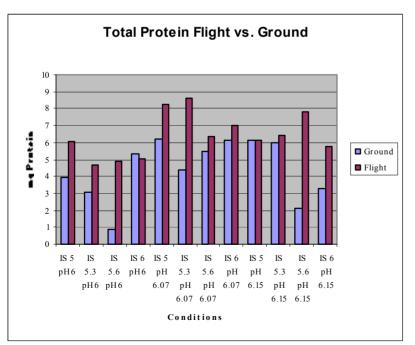
400X Photomicrograph / Polystyrene Micro spheres (1.4u)



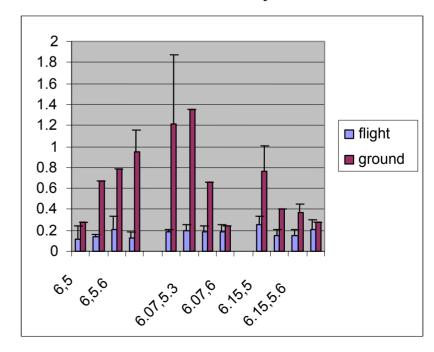
# Microgravity Interferon Crystallization Studies: STS-101 (Purification-Manufacturing)

- Temperature induced pI crystallization method
- Vary pH vs. salt
- Analyzed by HPLC and MS analysis: no difference between flight and ground crystals

Protein Assay Redissolved Crystals



Dynamic Light Scattering Analysis Redissolved Crystals



## STS-107 Experience

(Purification-Manufacturing)



- Follow-up experiment to STS-101
- Poisoned interferon with known impurities (proteineous and non-proteineous)
- Special mission (research based)
- Diversity of research experiments
- Type of mission NASA should continue in principle

## Summary

- Microgravity experimentation has allowed for advances in crystallization hardware and processing methods
- Partnering essential for success
- Value to overall research effort
- Future studies: ISS research tool (automation and micro fluidics)

# Acknowledgements

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